Signature: Med Sci Monit, 2003; 9(6): CR277-286
PMID: 12824951

CR

Received: 2003.04.25
Accepted: 2003.05.12
Published: 2003.06.25

An analysis of the link between polymorphisms of the beta2 and beta3 adrenergic receptor gene and metabolic parameters among Polish Caucasians with familial obesity

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
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Sources of support: The Polish Scientific Research Committee, Grants No: 4P05D 01016, 3P05D 08424 and 4P05D 05319. We are also grateful for help from a bilateral international co-operation grant between Poland and France, 'Polonium' No. 01801SJ and COST-918 Action

Summary

Background: Previous studies have suggested that genetic variation in the beta2 (β2-AR) and beta3 (β3-AR) adrenergic receptor genes are associated with obesity and insulin resistance. The aim of this study was to evaluate the influence of beta2 (Gln27>Glu) and beta3 (Trp64>Arg) adrenoreceptor gene polymorphisms on BMI and carbohydrate-lipid metabolism in Polish obese families.

Material/Methods: 122 persons (84 women, 38 men) from 40 obese families (BMI 33.5±7.7) were included. PCR-RFLP analysis of genotype was plotted against anthropometric parameters and the results of glucose and lipid oral tolerance tests. Venous blood samples were analysed for concentrations of glucose, insulin, free fatty acids, triglycerides, total cholesterol, HDL-chol, LDL-chol, leptin, and vWF.

Results: We found 39% Glu27 with 8% Arg64 allele frequencies. The blood glucose and insulin concentration during OGTT and blood FFA and TG level during OLTT was lower in patients with the Glu/Glu β2-AR polymorphism than Glu/Gln and Gln/Gln. In the obese patients the same effect was observed; however, the percent of fat body mass, leptin concentration, and BMI was higher in this group. Patients with the Trp/Trp polymorphism in the β3-AR gene were characterized by higher glucose and insulin concentration during OGTT and higher blood concentration of FFA and TG during OLTT. These results were independent of BMI value.

Conclusions: The β2-AR 27Glu and β3-AR 64Arg alleles have a protective effect against metabolic disorders in obese families from southern Poland.

key words: **β2-AR gene • β3-AR gene • obesity • metabolic disorders**

Full-text PDF: http://www.MedSciMonit.com/pub/vol_9/no_6/3648.pdf

Word count: 3663
Tables: 3
Figures: 9
References: 51

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BACKGROUND

It is well documented that both environmental and genetic factors are involved in the onset and progression of obesity in humans. Severe obesity appears to have a particularly strong genetic component and is polygenic in nature [1]. The sympathetic nervous system plays a key role in regulating the energy balance [2]. The adrenergic receptor genes are suggested to be the 'candidate genes' for obesity development, and for carbohydrate and lipid metabolism disorders.

The beta2 (β2-AR) and beta3 (β3-AR) adrenergic receptors are the main receptors involved in the regulation of thermogenesis and lipolysis in brown and white adipose tissue in rodents. Both β2-AR and β3-AR are expressed in human adipose tissue cells [3]. The beta-adrenergic receptors bind the endogenous catecholamines, transfer the signals to the interior of cells via the stimulatory guanine nucleotide–binding protein (Gs), and regulate basal metabolic rate (BMR) [4].

A comparison of the primary structures of beta-receptors confirms their remarkable degree of conservation, particularly in their transmembrane domains. There are, however, a number of species-related differences [5]. The structure of these receptors is characterized by the presence of seven hydrophobic regions, corresponding presumably to seven transmembrane domains [5]. The subtypes of beta-receptors appear to have an extracellular glycated N-terminal domain. In contrast to β1-AR and β2-AR, the C-terminal intracellular domain of β3-AR is apparently voided at the third intracellular loop, which is the phosphorylation target sequence of protein kinase A or beta adrenergic kinase [6]. Phosphorylation of β2-AR has been shown to be one of the first steps in receptor desensitization after agonist binding, thus the absence of the phosphorylation site may well explain resistance to short term desensitization [7].

The β3-AR is strikingly different from the β1 and β2-AR subtypes; it recognizes most of the β1 and β2-AR antagonists as agonists [6]. Another difference between β3- and β1- or β2-AR is that β3-AR reveals a lower affinity for catecholamines. This suggests that β1- and β2-ARs mediate the effect of circulating catecholamines, whereas the β3-AR mediates only the effects of much higher concentrations of norepinephrine [8].

A distinguishing feature of the β3-AR is that it appears to be relatively resistant to desensitization and down-regulation. This leads to the hypothesis that its primary function may be to maintain signaling during periods of sustained sympathetic stimulation [9].

Genes encoding the various adrenergic receptor subtypes may respond to different signals during ontogenesis. The majority of β3 specific mRNA is detected in brown adipose tissue, which in mammals other than rodents is found mainly in newborns or in pathological situations, such as pheochromocytoma, or rare climatic conditions (extreme cold) [6]. Isolated brown or white adipocytes found throughout the lifespan of human adults do, however, express β3-AR at the same time as they express β2- and β1-AR.

In adipocyte-like cells (3T3-F44-2A), the expression of β2-AR may be considerably up-regulated, and that of β1- and β3-AR almost completely suppressed by treatment with dexamethasone [5]. This up-regulation of β2-AR may be explained by the existence in the 5 flanking region of the β2-AR gene several glucocorticoid responsive elements consensus sequences, which are potential sites of interaction with the glucocorticoid receptor [5].

The β2-AR gene displays high genetic variability and common polymorphisms at codon, such as Arg16Gly or Gln27Glu (point mutation: C→G), and a mutation at codon 164 (Thr164Ile) could result in altered receptor function [10]. It has been suggested that the Glu27 variant is resistant to agonist-promoted down-regulation [11]. Although this variant has been associated with obesity [12,13] and type 2 diabetes [14,15], the findings have not been replicated in all studies [16,17]. Other authors have reported that polymorphisms in the β2-AR gene may influence the effects of physical activity [18] or diet [4] in the determination of body fat mass. In addition, the C to T nucleotide substitution at nucleotide 47 has been described in the 5' leader cistron (LC) of the β2-AR gene (5'LCAArg19-Cys), which is in linkage disequilibrium with the codon 16 and 27 polymorphisms [19].

A Trp64 Arg polymorphism in the first intracellular loop of the β3-AR gene (a non-conservative missense mutation, T→A) has been described in some studies as being associated with obesity and a variety of its related traits, such as high BMI and WHR, increased capacity to gain weight and decreased energy expenditure, insulin resistance and an earlier onset of type 2 diabetes [2,20,21]. These findings have been replicated in several [1,21–23] but not all studies [24]. The inconsistencies between studies have led some investigators to conclude that this polymorphism plays little if any role in human obesity [7].

The human β2-AR stimulates both lipolysis and fat tissue blood flow [25]. Evidence that β3-AR is expressed in visceral fat makes it a prime candidate for the regulation of lipolysis and insulin sensitivity in humans [26]. This receptor, by stimulating the uncoupling protein UCP-1, alters respiration coupling and dissipates oxidation-derived energy as heat [27]. Moreover, β3-AR-mediated effects are hypothesized to be modulated by leptin and vice versa [8].

The involvement of β2-AR and β3-AR in metabolic disorders suggests that polymorphism in the encoding genes might be an inter-individual susceptibility factor for these disorders and a myriad of disorders connected with them, i.e. breast cancer [28] and colon cancer [29]. Significantly, studies reported to date underline ethnic differences that imply region-specific polymorphism and its interaction with other obesity risk factors; for instance with polymorphisms of other obesity candidate genes, such as UCP-1 [10,30–32], D_2R [32,33] and the peroxisome proliferator receptor (PPAR) gamma [34],

physical inactivity, and a high carbohydrate/fatty diet. The strong impact of gender on the effects of polymorphism has also been proved in several studies [34].

The postulated role of the β2- and β3-ARs polymorphisms in obesity and its metabolic consequences prompted us to investigate the role of the Gln27Glu β2-AR and Trp64Arg β3-AR gene polymorphisms in members of obese families from Southern Poland.

MATERIAL AND METHODS

Our study was performed in 122 patients (38 men and 84 women) who belonged to 40 families with the genetic trait of obesity. Increased body weight (body mass index ≥30) was detected in at least two generations of the studied families. The subjects were recruited from outpatients seen at the Clinic of Lipid Disorders and Obesity in Cracow, Poland. All biochemical estimations were performed at the Department of Clinical Biochemistry, Jagiellonian University, Cracow, Poland. Patients with serious accompanying diseases (diabetes mellitus, cancer, inflammatory disease, symptomatic atherosclerosis, liver damage or any other disorder affecting metabolism) were excluded from the study. For at least two weeks before the study all patients consumed their usual diet, did not change their physical activity level, and did not take any medication that would influence the results of blood tests for lipids and carbohydrates. None had undertaken a weight-reducing effort during the most recent 6 months. All patients gave their informed consent to participate in the study, which was approved by the Ethics Committee of the Jagiellonian University in Cracow.

During the initial visit all patients were examined and office blood pressure was measured three times with a mercury sphygmomanometer after the patient had been seated for 10 minutes in a quiet room, according to ISH guidelines. A minimum of 3 readings were performed, and the average of the last 2 readings was recorded. Body mass index (BMI) was calculated with the usual formula, weight (kg)/height^2 (m). The waist was measured with a soft tape midway between the lowest rib and the iliac crest, and hip circumference at the widest part of the glutteal region. The waist-hip-ratio (WHR) was calculated as a measure of central adiposity. Body composition was determined by bioelectrical impedance (Maltron BF-905): percent body fat, measured as a percentage of total body weight and free fat mass, expressed as kilograms of free fat mass.

The laboratory blood tests, oral glucose tolerance test and oral lipid tolerance test were performed on different days. Blood samples were obtained after overnight fasting for genotyping and for estimation of blood concentration of glucose, insulin, total cholesterol (TCh), HDL, triglycerides (TG), free fatty acids (FFA), leptin, and von Willebrandt Factor (vWF).

For the oral glucose tolerance test (OGTT), a blood sample was drawn from a peripheral vein after overnight fasting, and again after ingestion of 75 g of glucose in a volume 300 ml every 30 minutes for 120 min. to measure blood glucose and insulin concentration.

The oral lipid tolerance test (OLTT) was performed according to Couderc, 1998 [35]. After a standard meal (energy intake of 1033 kcal, 40% fats (80 g), 20% proteins, 40% carbohydrate), blood samples were obtained at 2, 4, 6 and 8 hours to measure TG, FFA, leptin and insulin determination.

Blood **glucose** was measured by an enzymatic colorimetric method using glucose oxidase (Cormay Diagnostic, Poland). **Total cholesterol**, **HDL** and **triglycerides** were measured by an enzymatic method (Cormay Diagnostic, Poland). **FFA** concentration was measured by an optimized enzymatic colorimetric assay (Roche Diagnostic, Mannheim, Germany). Plasma **insulin** levels were estimated by immuno-radioassay (Polatom, Otwock, Poland). The serum concentration of **leptin** was assayed by radio-immunoassay kits (LINCO). The **vWF** concentration was determined by a commercial enzyme immunoassay kit (Diagnostica Stago, France).

Blood vWF estimation was also made in a group of 10 healthy volunteers (5 men, 5 women) aged 48±7.9 years as a control for the measurements performed in patients from obese families.

Insulin resistance was evaluated using several indexes:
- HOMA-IR (homeostasis model assessment of insulin resistance) according to the equation:
  HOMA-IR = [Fasting insulin(mikroU/mL)×(fasting glucose(mmol/l)]/22.5 [36],
- DELTA (early secretory response to an oral glucose load) according to the equation:
  DELTA= [DELTA I30-I0 (pmol/L)] / DELTA G30-G0 (mmol/L)] [37],
- AUC-: the computed area under the curve, expressed the glucose, insulin, FFA and leptin concentrations during tests.

Genomic DNA was isolated from whole blood using QIAamp Blood and Tissue Kits (Qiagen Inc, Germany). The Trp64Arg β3-AR gene polymorphism was determined by polymerase chain reaction (PCR) performed with 20 ng of genomic DNA with upstream primer 5' CCA GTG GGC TGC CGA GGG 3' and downstream primer 5' GCC AGT GGC GCC CAA CGG 3'. The resulting 248 bp product was digested with *Mva I* (Amersham Pharmacia Biotech). The digested products were subjected to electrophoresis through a 3% agarose gel. The gel was stained with ethidium bromide and DNA was visualised by UV transillumination. The presence of two restriction sites (Trp64 allele) resulted in fragments of 97, 61 and 64 bp, and the loss of one restriction site (Arg64allele) resulted in fragments of 158 and 64 bp.

The primers that were used to amplify simultaneously codon 27 for measurement of the Gln27Glu polymorphism were derived from the genomic sequence of the β2-AR gene. The forward primer was 5' GAA TGA GGC TTC CGA GCG TC 3' and the reverse primer was 5' GGC CCA TGA CCA GAT CGA CA 3' resulting in a

Table 1. Characteristics of the study group (*p<0,05; **p<0,001).

	Whole group (n=122)		Female (n=84)		Male (n=38)		p
	Aver.	SD	Aver.	SD	Aver.	SD	Male vs. female
Age [years]	43.55	19.15	46.46	18.44	37.13	19.36	0.012*
BMI [kg/m^2]	33.26	7.67	33.67	7.35	32.36	8.36	0.383
WHR	0.87	0.1	0.84	0.08	0.93	0.09	0.000**
Lean body mass%	57.38	11.79	51.61	7.98	68.30	10.01	0.000**
Fat body mass %	37.92	16.9	36.39	14.37	40.81	20.86	0.274
Diastolic BP [mmHg]	80.7	11.8	80.29	10.90	81.74	14.03	0.622
Systolic BP [mmHg]	129.22	14.52	129.08	13.36	129.57	17.45	0.892
vWF [%]	120.94	43.42	119.13	40.19	124.35	49.40	0.568
Fasting glucose[mmol/l]	5.50	0.92	5.51	0.98	5.48	0.79	0.890
Fasting insulin [μU/ml}	15.78	9.97	15.71	10.23	15.92	9.61	0.926
TCh [mmol/l]	5.18	1.21	5.21	1.18	5.13	1.28	0.749
HDL [mmol/l]	1.34	0.32	1.39	0.32	1.24	0.29	0.017*
LDL [mmol/l]	3.09	1.01	3.05	1.08	3.17	0.82	0.591
Leptin [pg/ml]	22.59	14.69	27.41	12.38	12.57	14.26	0.000**
HOMA- IR	3.95	2.84	3.97	3.09	3.91	2.33	0.925
DELTA	849.64	5284.64	1153.08	6524.15	272.12	184.29	0.455

353 bp product size. The expected sizes after digestion with *ITA I* (Amersham Pharmacia Biotech) were 174, 97, 55 and 27 bp for Gln27 homozygotes; 229, 97 and 27 bp for Glu27 homozygotes; and 229, 174, 97, 55 and 27 bp for heterozygotes.

Statistical analysis

The results of continuous variables are expressed as means ± SD. Before statistical analysis, normal distribution and homogeneity of variables were tested. We used the χ^2 test for comparisons of proportions and the unpaired t test for comparisons of quantitative variables. The levels of statistical significance were set at $p<0.05$. The statistical analysis was performed with the Statistica for Windows software from Statsoft.

RESULTS

The characteristics of the studied patients are given in Table1. The females were significantly older than the males. There was no difference in BMI between the men and the women, but the WHR ratio was higher in the men.

The mean value of blood leptin was significantly higher in the women than in the men.

The whole study group of patients revealed a higher (but not significantly higher) blood concentration of vWF (120.9%±43.4) as compared to the 10 healthy volunteers (80.6%±41.2).

The distribution of genotypes was consistent with the population, in Hardy-Weinberg equilibrium, and not significantly influenced by gender and obesity status.

The genotype distribution of the β2-AR Gln27Glu polymorphism in the whole group was 42%, 40 % and 18% for the Gln27Gln, Gln27Glu and Glu27Glu genotypes respectively. The genotype distribution of the β3-AR in the study group was 86%, 13% and 1% for Trp64Trp, Trp64Arg and Arg64Arg respectively.

The allele frequency of β2-AR Glu27 was 39%, and β3-AR Arg64, 8%.

The blood glucose concentration in all patients with the Glu/Glu β2-AR polymorphism was lower than in Glu/Gln and Gln/Gln carriers during the entire OGTT (significantly at 90 min after glucose ingestion) (Figure 1). The serum insulin concentration was also the lowest in subjects with the Glu/Glu polymorphism, but not significantly (Figure 2).

In the group of patients with BMI <30 kg/m^2 there were no differences in blood glucose and insulin concentration during OGTT among different Glu27 β2-AR allele carriers. In the obese patients (BMI>30 kg/m^2, the subjects with the Glu/Glu polymorphism revealed a tendency to lower concentration of glucose and insulin measured in blood during OGTT, although the percent of fat body mass and BMI was the highest in this group (Table 2). In the group of men with Glu/Glu polymorphism (n=5), the leptin level was 28.53 ng/ml and was significantly higher ($p<0.001$) than in the men with Gln/Gln (10.19 ng/ml) and Gln/Glu (13.16 ng/ml).

The blood concentrations of TG and FFA measured during OLTT were the highest in the group of Gln/Gln β2-AR carriers, while the lowest TG concentrations were observed in patients with Glu/Glu polymorphism; however, the differences were not significant (Figure 3,4).

The obese men (BMI≥30 kg/m^2) with Gln/Gln polymorphism revealed a higher concentration of vWF ($p<0.05$) compared to obese carriers of Gln/Glu (Table 2).

The patients with Trp/Trp polymorphism in the β3-AR gene were characterized by an insignificantly higher glucose concentration in comparison to Arg carriers during the whole OGTT (Figure 5).

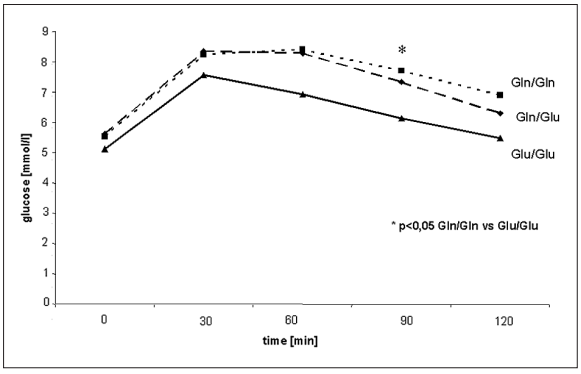

Figure 1. Glucose concentration during OGTT in patients with polymorphism β-2AR.

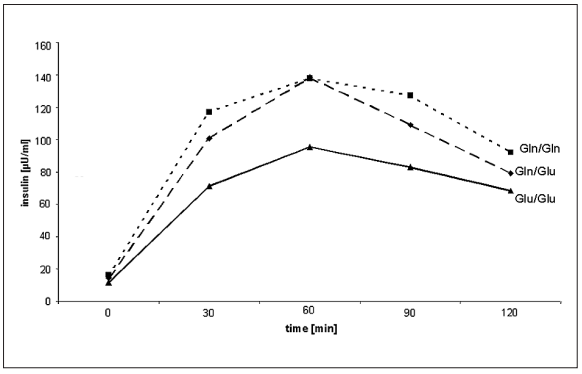

Figure 2. Insulin concentration during OGTT in patients with polymorphism β-2AR.

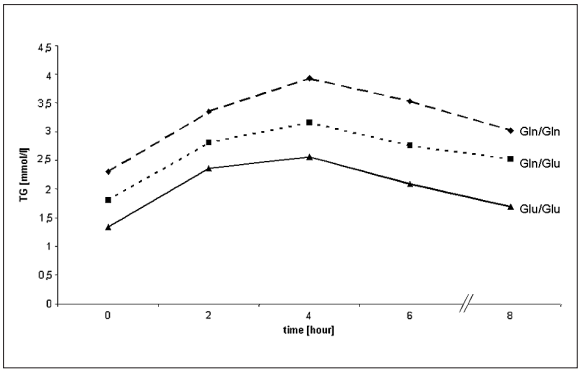

Figure 3. Tryglicerides concentration during OLTT in patients with polymorphism β-2AR.

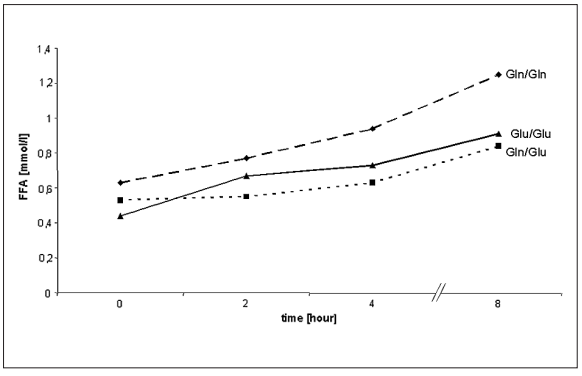

Figure 4. Free facid acid concentration during OLTT in patients with polymorphism β-2AR.

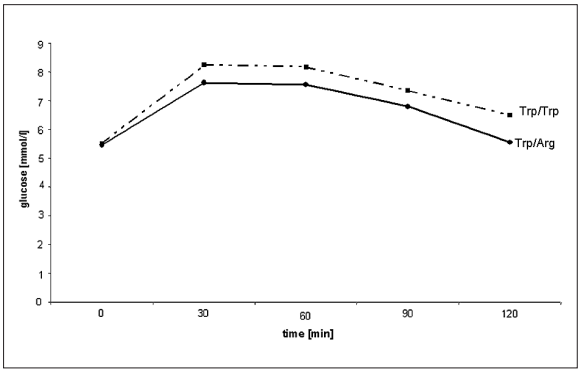

Figure 5. Glucose concentration during OGTT in patients with polymorphism β-3AR.

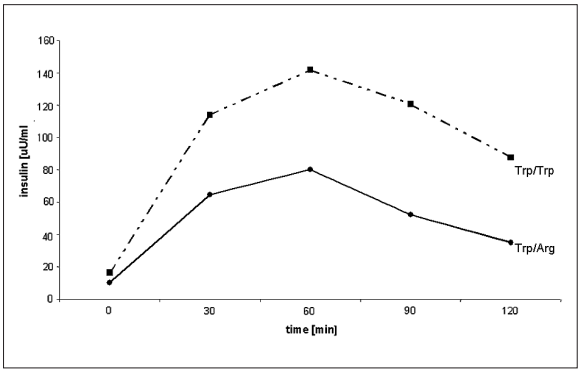

Figure 6. Insulin concentration during OGTT in patients with polymorphism β-3AR.

Insulin concentration during the whole OGTT was also higher among patients with Trp/Trp polymorphism (Figure 6), with statistically significant results at 90 min into the OGTT.

The group of men with BMI<30 kg/m^2 and the Trp/Trp variant had lower glucose concentration (significantly at 60 and 90 min of the OGTT and AUC Glu) and slightly lower AUC Ins value during OGTT, which argues for better glucose tolerance (Table 3).

An analysis of the OLTT results shows higher blood concentration of Tg (Figure 7) and FFA (Figure 8) in the group of Trp/Trp carriers. The blood concentration of insulin at 0, 2, 6, and 8 hours into the test was higher among patients with the Trp/Trp polymorphism (Figure 9).

Discussion

The results of our study suggest that the Arg64 variant of β3-AR gene is not frequent in the population of southern Poland. The second investigated mutation of

Table 2. The phenotypic and metabolic characteristics of study group males during OGTT, grouped according to BMI value and Gln/Glu status (polymorphism at position 27 in the β2-AR gene); *p<0,05.

OGTT parameter	Male group I (BMI<30)							
	Gln/Gln (n=8)		Gln/Glu (n=6)		Glu/Glu (n=2)		p	p
	Ave.	SD	Ave.	SD	Ave.	SD	Gln/Gln vs. Gln/Glu	Gln/Gln vs. Glu/Glu
Age [years]	31.60	26.82	30.31	21.40	35.71	24.45	0.925	0.850
BMI [kg/m^2]	23.00	4.11	26.08	0.80	27.50	2.12	0.098	0.184
WHR	0.82	0.10	0.88	0.04	0.86	0.04	0.184	0.639
Lean body mass%	61.50	2.12	56.50	9.40	61.00	0.00	0.521	0.879
Fat body mass %	18.00	5.66	21.75	3.10	24.00	0.00	0.329	0.546
Diastolic BP [mmHg]	80.00	14.14	74.00	5.48	70.00	0.00	0.407	0.572
Systolic BP [mmHg]	127.50	12.58	124.00	8.94	110.00	0.00	0.639	0.302
vWF [%]	123.82	48.06	91.32	27.98	147.45	38.54	0.217	0.557
O Glucose 0 [mmol/l]	5.17	0.80	4.83	0.39	5.20	0.47	0.415	0.970
G Glucose 30 [mmol/l]	7.23	1.66	7.25	0.68	6.86	0.72	0.983	0.783
T Glucose 60 [mmol/l]	6.83	0.85	6.88	2.15	7.69	2.26	0.963	0.457
T Glucose 90 [mmol/l)]	5.83	0.88	6.15	1.57	6.53	1.19	0.698	0.415
Glucose 120 [mmol/l]	5.25	1.03	4.74	1.30	4.55	1.16	0.511	0.467
Insulin 0 [μU/ml]	7.30	3.59	12.00	6.28	9.40	2.83	0.198	0.500
Insulin 30 [μU/ml]	60.84	41.39	83.58	61.79	82.00	48.79	0.529	0.582
Insulin 60 [μU/ml]	76.58	54.69	93.18	78.48	86.20	17.82	0.719	0.826
Insulin 90 [μU/ml]	46.22	21.00	76.20	35.41	91.40	16.40	0.156	0.044*
Insulin 120 [μU/ml]	35.14	12.67	31.03	17.73	24.75	12.80	0.696	0.373
AUC Glu	752.88	68.41	751.86	131.16	778.43	106.17	0.988	0.710
AUC Ins	6145.80	3098.31	8233.88	5082.68	8300.25	1356.58	0.469	0.406
HOMA-IR	1.77	1.08	2.57	1.48	2.14	0.46	0.378	0.670
DELTA	191.86	86.17	227.01	174.31	325.66	35.67	0.702	0.098
OGTT parameter	**Male group II (BMI⩾30)**							
	Gln/Gln (n=13)		Gln/Glu (n=6)		Glu/Glu (n=3)		p	p
	Ave.	SD	Ave.	SD	Ave.	SD	Gln/Gln vs. Gln/Glu	Gln/Gln vs. Glu/Glu
Age [years]	46.52	13.98	38.45	15.19	23.13	9.52	0.270	0.017*
BMI [kg/m^2]	36.70	5.66	38.50	4.28	42.00	11.14	0.500	0.239
WHR	0.98	0.04	1.01	0.08	0.97	0.11	0.365	0.785
Lean body mass%	71.25	8.09	72.80	5.97	71.67	16.26	0.706	0.948
Fat body mass %	39.92	11.86	54.60	10.45	67.67	41.06	0.030*	0.046*
Diastolic BP [mmHg]	83.75	11.88	86.67	15.28	95.00	35.36	0.742	0.420
Systolic BP [mmHg]	131.25	16.42	136.67	25.17	140.00	42.43	0.679	0.620
vWF [%]	112.60	41.77	166.00	63.00	132.70	59.52	0.041*	0.495
O Glucose 0 [mmol/l]	5.92	0.84	5.92	0.54	4.82	0.08	0.991	0.000*
G Glucose 30 [mmol/l]	9.16	1.50	9.33	1.78	8.15	2.73	0.844	0.395
T Glucose 60 [mmol/l]	10.71	2.68	9.31	2.22	7.42	1.61	0.298	0.072
T Glucose 90 [mmol/l)]	9.11	2.34	6.96	1.50	5.98	1.63	0.061	0.053
Glucose 120 [mmol/l]	7.70	3.13	5.22	1.77	4.78	1.58	0.095	0.151
Insulin 0 [μU/ml]	19.03	8.87	18.13	4.21	24.03	20.84	0.821	0.525
Insulin 30 [μU/ml]	111.43	53.54	176.23	64.72	121.87	51.98	0.047*	0.769
Insulin 60 [μU/ml]	219.92	93.82	204.77	112.31	139.93	63.87	0.775	0.200
Insulin 90 [μU/ml]	175.95	82.90	108.25	44.03	65.70	20.27	0.085	0.046*
Insulin 120 [μU/ml]	117.82	97.72	35.08	27.56	123.23	151.15	0.064	0.940
AUC Glu	1079.04	243.11	934.65	171.46	790.50	192.34	0.225	0.089
AUC Ins	17804.25	7228.19	15475.75	4018.16	12034.00	4533.60	0.484	0.225
HOMA-IR	4.89	2.24	4.76	1.15	5.13	4.44	0.898	0.896
DELTA	208.47	97.11	401.30	238.30	405.39	370.48	0.000*	0.110

the β2-AR gene is more frequent, which points to the role of ethnic differences in the frequency of the obesity related 'gene candidate' polymorphisms [1,23,25,32]. We found no significant relations between the investigated polymorphisms of AR genes and BMI values; however, we observed statistically significant links between these polymorphisms and some metabolic parameters among the members of obese families.

Focusing on the investigated β3-AR polymorphism, it should be noted that two of the β3-AR 64 Arg allele carriers represented the highest BMI value, approaching

Table 3. The phenotypic and metabolic characteristics of study group males during OGTT, grouped according to BMI value and Trp/Arg (polymorphism at position 64 in the β3-AR gene); *p<0,05.

OGTT parameter	Male group I (BMI<30)				
	Trp/Trp (n=14)		X/Arg (n=2)		p
	Ave.	SD	Ave.	SD	Trp/Trp vs. Trp/Arg
Age [years]	25.44	16.77	74.96	4.29	0.001*
BMI [kg/m^2]	24.39	3.55	27.00	0.00	0.331
WHR	0.85	0.07	0.93	0.00	0.270
Lean body mass%	59.83	6.97	51.00	0.00	0.293
Fat body mass %	20.83	4.22	22.00	0.00	0.808
Diastolic BP [mmHg]	77.50	10.35	70.00	0.00	0.356
Systolic BP [mmHg]	122.50	11.65	130.00	0.00	0.409
vWF [%]	119.51	40.44	89.90	60.81	0.386
O Glucose 0 [mmol/l]	4.93	0.42	5.55	1.25	0.185
G Glucose 30 [mmol/l]	6.85	0.65	8.78	1.89	0.016*
T Glucose 60 [mmol/l]	6.60	1.20	9.00	2.27	0.044*
T Glucose 90 [mmol/l)]	5.94	0.89	6.75	2.63	0.400
Glucose 120 [mmol/l]	4.86	0.90	5.22	2.42	0.691
Insulin 0 [μU/ml]	9.62	5.04	8.35	4.60	0.752
Insulin 30 [μU/ml]	71.91	45.12	77.65	75.45	0.885
Insulin 60 [μU/ml]	78.19	53.28	112.15	82.38	0.467
Insulin 90 [μU/ml]	66.73	33.34	59.05	21.57	0.767
Insulin 120 [μU/ml]	30.16	14.13	38.95	13.51	0.444
AUC Glu	728.64	67.51	897.08	107.87	0.013*
AUC Ins	7101.67	3747.82	8175.00	3954.14	0.724
HOMA-IR	2.11	1.14	2.19	1.60	0.939
DELTA	248.48	118.37	141.18	129.57	0.281
ISI-Comp	6.06	3.69	4.65	2.50	0.627
OGTT parameter	**Male group II (BMI⩾30)**				
	Trp/Trp (n=21)		X/Arg (n=1)		p
	Ave.	SD	Ave.	SD	Trp/Trp vs. Trp/Arg
Age [years]	40.16	15.23	61.41	0.00	0.188
BMI [kg/m^2]	38.29	6.04	30.00	0.00	0.195
WHR	0.99	0.06	0.99	0.00	0.936
Lean body mass%	72.32	8.35	60.00	0.00	0.168
Fat body mass %	48.74	19.93	29.00	0.00	0.347
Diastolic BP [mmHg]	85.83	16.21	90.00	0.00	0.810
Systolic BP [mmHg]	135.00	21.11	120.00	0.00	0.509
vWF [%]	128.45	54.14	160.40	0.00	0.571
O Glucose 0 [mmol/l]	5.72	0.79	6.44	0.00	0.384
G Glucose 30 [mmol/l]	9.08	1.77	8.64	0.00	0.810
T Glucose 60 [mmol/l]	9.75	2.59			
T Glucose 90 [mmol/l)]	8.00	2.40	7.97	0.00	0.991
Glucose 120 [mmol/l]	6.48	2.91	7.19	0.00	0.816
Insulin 0 [μU/ml]	20.01	9.79	10.00	0.00	0.332
Insulin 30 [μU/ml]	136.31	60.56	58.90	0.00	0.229
Insulin 60 [μU/ml]	202.51	95.82			
Insulin 90 [μU/ml]	141.79	79.33	87.90	0.00	0.516
Insulin 120 [μU/ml]	95.97	97.99	52.80	0.00	0.673
AUC Glu	987.88	232.13			
AUC Ins	16157.84	6114.52			
HOMA-IR	5.00	2.26	2.86	0.00	0.370
DELTA	303.04	213.42	159.48	0.00	0.520
ISI-Comp	2.04	0.82			

40 kg/m^2 in our group. Regardless of the obesity phenotype, the mutated allele carriers presented with physiological lipid parameters (high HDL, low FFA), good lipid tolerance during OLTT, as well as good glucose tolerance parameters. Statistically their levels of insulin and the insulin resistance index (ISI-HOMA) were significantly decreased, pointing to good insulin sensitivity.

Thomas et al. in 1999 [12] suggested the possibility of coexistence of the Trp64Arg β3-AR polymorphism and a form of visceral obesity with decreased lipolysis. The

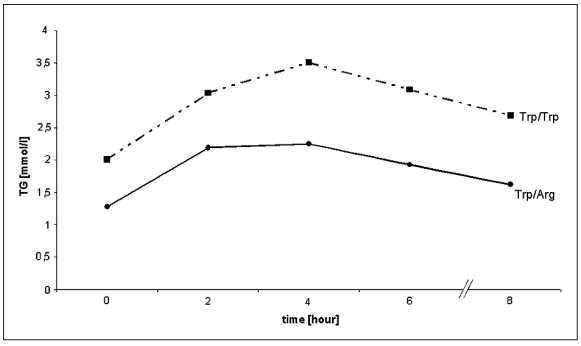

Figure 7. Tryglicerides concentration during OLTT in patients with polymorphism β-3AR.

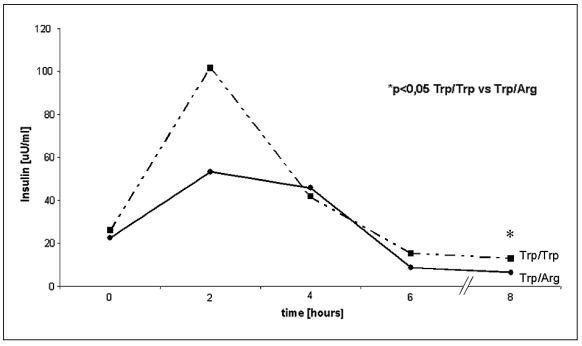

Figure 8. Tryglicerides concentration during OLTT in patients with polymorphism β-3AR.

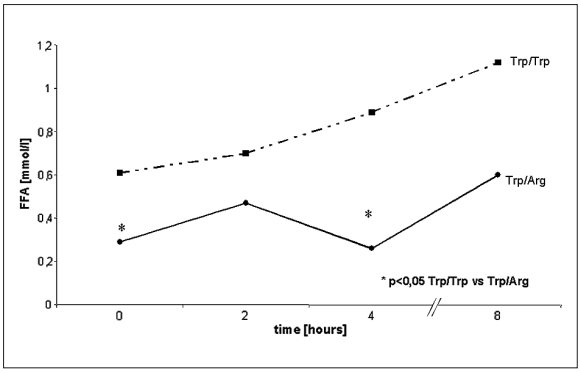

Figure 9. Free fatty concentration during OLTT in patients with polymorphism β-3AR.

mutated allele has also been associated with impaired catecholamine-induced lipolysis in other studies [38–40]. The mechanism by which this may influence FFA and other parameters of glucose/fat metabolism is still not clear. A lower FFA concentration seems to protect against consequences leading to metabolic disorders. As postulated, FFA stimulates hepatic glucose and lipoproteins production [41,42] and interferes with insulin extraction [43]. By substrate competition in the muscle, higher FFA concentrations can lead to impaired insulin-stimulated glucose metabolism and insulin resistance. In the pancreas, increased FFA concentrations induce beta-cell dysfunction. In our group of patients, the low level of FFA may be related to low activity of lipolysis in mutated β3-AR allele carriers. This mutation may, in this way, be responsible for relatively normal glucose tolerance in spite of obesity in our group.

Several reports [44–46] have failed to replicate in humans the original reports indicating an association between the Arg64 allele in β3-AR and weight gain, insulin resistance and diabetes [43,47,48]. One potential reason for discrepancies among investigators is that this genetic variant may interact with the other genetic variants (polymorphisms) influencing body fat [49].

A thrifty gene haplotype is an hypothesis also worth mentioning. The β3-AR 64Arg allele seems to be a thrifty gene candidate as it is more frequent among the women and is associated with menarche at earlier age, and with a decrease in energy expenditure [40].

When the results for mutated β-3AR Glu27 carriers are analyzed, it is evident that there exists a higher frequency of this allele among the non-obese women (BMI<30) in our group. Homozygotic carriers of this allele with BMI>30 were characterized by lower glucose and insulin level during the OGTT test, as well as low TG level during the OLTT test, pointing to the low frequency of metabolic, or at least carbohydrate catabolic complications.

Evidence for the role of β2-AR in the etiology of obesity has been explored in recent studies. This mutation has been reported to be associated with obesity in Japanese men and women [13,14]. Swedish women homozygous for Glu27 had an average fat mass in excess of twenty kg and approximately 50% larger fat cells than women homozygous for Gln27. There have been no significant associations observed concerning changes in β2-AR function, as assessed by in vitro fat cell lipolysis experiments [12].

Statistically, in our study non-obese men with BMI <30 had a significantly lower level of FFA and increased level of leptin. The hypothesis which emerges from our results is that the lipolysis may be more efficient in β2-AR Glu27 carriers than in Gln27 carriers, leading to changes in lipolytic parameters. As efforts to analyze the activity of Glu27 isoform indicate, it does not reach the mature wild type conformation. This may result in an altered ability to be degraded, with metabolic consequences [10].

In another stratification, the subgroup of obese men (BMI>25) carrying this mutation was characterized by lower glucose, insulin, LDL and TG levels. However, the men in this group were younger in comparison to non-carriers of the mutation. The results may be due to the differences in age, since aging is known to reduce the adrenergic receptor beta sensitivity [50,51].

Our results are in line with a Swedish study [35], in which obesity in males tended to be negatively associated with the β2-AR 27Glu mutation. The genetic factors contributing to obesity are different between men and

women. Moreover, it has been shown that in Japanese subjects the frequency of the β2-AR Glu27allele in obese was higher than in non-obese subjects [13,14]. However, the frequency of the Glu27 allele in non-obese Japanese for both genders was much lower compared with that found in French or Swedish subjects. This difference may partially explain the differences in metabolic rates observed between Japanese and European subjects [18].

It has also been shown that physical activity, another compensation mechanism of lipid and glucose tolerance, was able to counterbalance the effect of β2-AR Gln27Glu polymorphism to increase body weight, body fat and obesity in men [18]. However, since we did not test the physical activity of our subjects, we cannot address this issue.

Depending on group stratification according to BMI value (we used cut-off values of 25 or 30), the obesity results were significantly different; thus supporting the well-known influence of obesity per se on metabolic parameters.

The introduced stratification related to blood pressure value revealed statistically significant higher insulin level among men-carriers of β2-AR 27Gln in comparison to non-carriers of this allele. As there were no more significant associations, this is unlikely to implt any linkage of the examined polymorphisms and susceptibility to hypertension. This issue has also been studied in other populations [51].

CONCLUSIONS

We found that the presence of polymorphisms of the β2- and β3-AR genes in our population is not directly related to obesity. Our results even revealed the protective effect of β2-AR 27Glu and β3-AR 64Arg alleles on metabolic (lipid and glucose tolerance) parameters in obese families of Southern Poland.

Acknowledgments

We would like to thank Anna Gruca, Anna Zdzienicka, Teresa Krzeszowska and Aneta Sobesto from the Department of Clinical Biochemistry, Collegium Medicum, Jagiellonian University, for excellent technical assistance and experienced technical advice.

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